EXHIBIT A

Carrageenan
Nature's Most Versatile Hydrocolloid

HENRY J. WITT
Marine Colloids
Rockland, Maine 04841

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CARRAGEENAN, NATURE'S MOST VERSATILE HYDROCOLLOID

INTRODUCTION

Carrageenan, as most of you know, is a generic term referring to the family of water soluble sulfated galactan extracts of certain types of red seaweeds or macroalgae (Rhodophyceae). The name came from the County Carrageen on the coast of Ireland where *Chondrus crispus*, better known as Irish Moss, was an article of commerce in the 1800's. Since then, "carrageenan" bearing weeds have been discovered all over the world, from frigid waters of the Maritime Provinces and New England to tropical lagoons in the Philippines. Commercial quantities are also harvested from the shores of Spain, France, Argentina, Chile and Korea. In the Philippines, *Eucheuma spinosum* and *Eucheuma cottonii* are now farmed as a result of the cooperative efforts of educational, governmental, and industry groups in the Philippines and the United States.

Carrageenan is similar in some respects to the animal mucopolysaccharides such as heparin and chondroitin sulfate, and exhibits many of the same properties including anti-coagulant activity. The key to carrageenan activity is molecular weight. Products are available in the range from roughly 10,000 to one million daltons. In general, most carrageenan products are in the range from 100,000 to 500,000 daltons. Carrageenan finds wide use in diverse applications including: toothpaste, ice cream, chocolate milk, low calorie jellies, milk puddings, pet foods, pharmaceutical and industrial suspensions, anti-ulcer treatment, shampoos, creams, lotions, o/w and w/o emulsions and many others.

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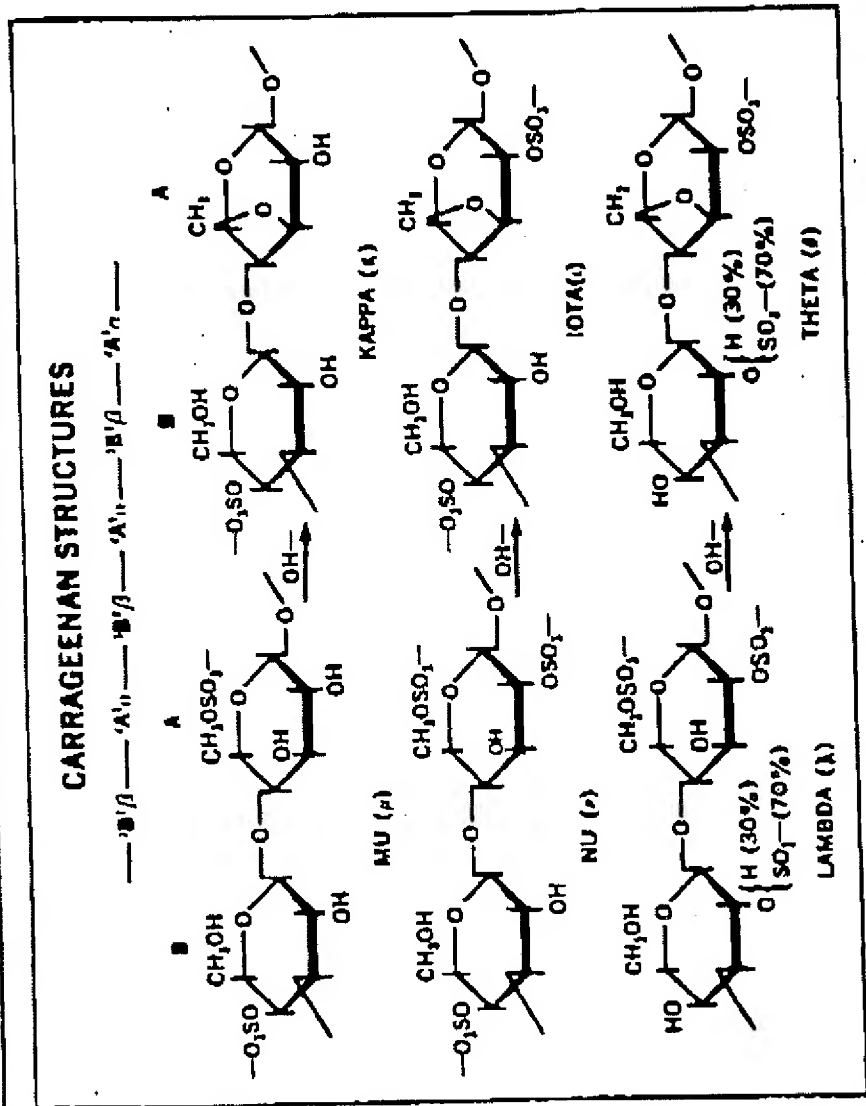

Figure 1: The Fractions of Carrageenan

As you can see from Figure 1, there are three pairs or six major fractions representing idealized structures of carrageenan. In nature, they exist in combinations. A balance of one or more pairs are present in each plant type. The fractions on the left (mu, nu, and lambda) can be converted to their corresponding fraction (kappa, iota or theta) by alkaline or enzyme modification, which closes the 3,6-anhydro ring. As I said, a given plant type can contain one or more pairs. These pairs are found by themselves and mixed, individual molecules, or as combinations in the same molecule. One common combination is kappa with iota moieties, and is referred to as kappa-2.

Aqueous Properties. Carrageenans, depending on type, form ion dependent gels or impart viscosity to aqueous solutions. In general, carrageenans exhibit moderate to relatively low water viscosities. Most commercial products fall in the range from 25 to 500 mPa·s with the majority in the range from 25 to 100. Native lambda, however, can develop viscosities as high as 20,000 mPa·s. Unfortunately, pure lambda-bearing weeds are commercially unavailable and even if they were, it is uneconomical to recover such high viscosity materials by alcohol precipitation, or any other means we are presently aware of.

The water gelation properties of carrageenan[2] are even more varied. Unmodified fractions, mu, nu and lambda, along with theta, are nongelling. However, completely unmodified fractions exist only in theory leaving lambda theta materials as the only truly non-

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gelling carrageenan. On the other hand, the <u>kappa</u> and <u>iota</u> fractions form strong water gels at concentrations as low as 1% and less. <u>Kappa</u> gels are firm, brittle and synerese, or exude water, particularly when cut. <u>Iota</u> gels, by contrast, are flexible, resilient, and very dry. The combination of these happy circumstances, allow the formation of water gels with just about any properties desired ranging from sparklingly clear water dessert gels and low calorie jellies to air freshener gels and cell-immobilization media[3]. <u>Iota</u> carrageenan forms gels at concentrations as low as 0.3% (w/v); <u>kappa</u> carrageenan will gel as low as 0.5% (w/v).

Carrageenan water gels are thermally reversible and are usually cast from a hot solution and cooled to set. However, they can also be chemically set similarly to alginate. Taking advantage of the fact that the sodium salts of <u>kappa</u> and <u>iota</u> are cold water soluble, solutions can be made without heat and set by the addition of calcium in the case of <u>iota</u>, and potassium with <u>kappa</u> (usually 3 to 5% of the weight of the carrageenan). Cell immobilization techniques use this method to form gels, avoiding the use of lethally high temperatures which would be otherwise required. Carrageenan gels can also be set with other cations including sodium but substantially higher concentrations of "salt" are needed.

Low concentration (0.5% to 1.0%) <u>iota</u> gels are additionally interesting, because they can be easily broken forming a thixo-

tropic sol characterized by a low yield point. The result is an extremely effective suspension medium. Very dense particles such as cerium oxide, have been suspended permanently in a readily pourable state.

Freeze-thaw properties of carrageenans are also varied. Iota carrageenan gels are generally unaffected by freeze-thaw cycling. Kappa carrageenan gels, on the other hand, fracture and synerese. In fact, this method, the "gel" press technique, has been employed to produce kappa carrageenan, analogous to agar-agar. The same method will also fractionate kappa from lambda the same as agar-agar is separated from agaropectin. Iota carrageenan, naturally, cannot be concentrated in this manner.

Lambda carrageenan, although nongelling, has excellent freeze-thaw properties in that its solubility is not affected by freezing. As a result, products containing lambda are used extensively as freeze-thaw stabilizers. Carrageenan has also been found effective in preventing the migration of substances such as colors, flavors, and even antibiotics in frozen products such as ice-pops and commercial freezer ice. Other common applications include frozen coffee whiteners, natural and imitation whipped cream, and pizza sauces.

Because of the high charge density of carrageenan it acts as a dispersant, particularly in very low molecular weight form. Standard commercial products also have this property. At low con-

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centrations in chocolate drinks, carrageenan prevents the cocoa from hard packing, allowing easy redistribution. At higher levels the carrageenan will suspend the cocoa permanently in both dairy and nondairy systems. At the same time, the carrageenan prevents the separation of any fats which may be present. This ability to disperse has been applied to pigments and insoluble salts such as barium sulfate used in X-ray contrast media[5].

The high molecular weight, anionic nature and long chain lengths of carrageenan have beeb employed to aid in the precipitation of such moieties as proteins[6] and lactose from whey, proteins from waste waters[8] and even common salt[7].

<u>Protein Reactivity</u>. Undoubtedly, the most interesting and useful aspect of carrageenan functionality is its protein reactivity. Generally, when a protein's molecular configuration is open or "denatured," carrageenan will react with it. The degree of reaction will depend on a number of conditions. First, the type of carrageenan, that is the degree of sulfation or fractional type, the molecular size and the pH of the system. Above the isoelectric point of the protein, the reaction is generally mild resulting in increased viscosity or gelation. At or below the isoelectric point of the protein the reaction is strong invariably resulting in coprecipitation. Both conditions are very useful as we will see.

MILK SYSTEMS

Hanson[9] explained the mechanism of the reaction between kappa carrageenan and kappa casein - whereby the entire casein micellular structure is stabilized. Iota and lambda carrageenans also react with milk proteins with similar but different results from kappa. As a result, the fractional distribution of any carrageenan product is critical to its performance in milk applications in particular. This is seen very clearly in the performance differences between Chondrus crispus and Eucheuma cottonii extractives. Chondrus is composed of about 65% kappa fraction with about 5% iota moieties, the balance being lambda. Cottonii extracts, on the other hand, are pure kappa. While Chondrus-derived products are used universally in milk applications, cottonii, with one exception, is rarely used.

The exception, discovered by Guiseley[10], teaches that kappa carrageenan within narrow viscosity range of from about 7 to 10 mPa·s's exhibits outstanding milk properties in that it does not gel milk even at relatively high concentrations of the order of 0.1 to 0.2% As a result, the material can be used in applications such as flavored milks, ice cream, etc. at concentrations many times higher than other carrageenan products. This is particularly useful to processors who have plant-to-plant variability problems or want their products to have high viscosities without gumminess. The explanation of this phenomenon is that as one reduces the molecular size of a carrageenan, water gel strength decreases while milk reactivity increases at the critical point -

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7 to 10 mPa·s - the two competing reactions interfere with each other resulting in a much diminished milk gel capability.

The measure of the effectiveness of carrageenan when used in milk systems is the level at which it is used. In puddings and pie fillings use levels are in the range of 0.3% compared to 3% or more for starch and gelation. In chocolate milk, carrageenan suspends cocoa and prevents fat separation at use levels around 0.025% (250 ppm). In commercial ice cream it prevents whey separation due to the presence of other stabilizers at levels of about 150 ppm. Finally, in evaporated milk, it stabilizes the fat with as little as 50 ppm.

It seems appropriate at this point to describe how carrageenan stabilizes milk fat. As you probably know, milk fat globules posses a surface coating of nature's emulsifier, the protein-like lecithin. It is generally believed that carrageenan reacts with the lecithin, keeping the associated fat globules in suspension. As a matter of fact, milk products containing carrageenan do not require homogenization.

Carrageenan milk gels are generally described as light and creamy. They resemble egg custards and flans in appearance and eating qualities. In fact, commercial egg custards and flans are invariably made with carrageenan rather than eggs. They are extremely popular just about everywhere outside the United States, where starch puddings are King. However, even here, the higher quality

pudding mixes contain some carrageenan. Its presence gives the finished product a lighter, creamier texture, as might be expected. It also provides a measure of insurance in that the carrageenan will "set" the pudding whether or not the starch has been properly cooked.

As is the case with water gels, kappa and iota carrageenan milk gels are quite different. Kappa milk gels are firm with some syneresis while iota milk gels are elastic, very much like egg custard. but with no syneresis. Lambda does not gel milk in the same way as kappa and iota, rather it imparts a creamy, "mousse-like" texture and mouthfeel. As a consequence, one has a great deal of latitude in designing those milk-based dessert products.

Generally speaking, carrageenan milk products, including puddings, flans, chocolate milks, etc., require heating and cooling to solubilize the kappa and iota carrageenan. The exception to this is lambda[12] which is soluble and reactive with cold milk. The limiting factors are dispersion and availability. The better one can disperse a lambda carrageenan product the more rapidly it will hydrate. Sounds simple, but is it? First, the product needs to be ground as finely as possible. Grinding down to -270 mesh is about as far as we can go. It's good, but finer would be better. Next, processing, such as agglomeration with other materials such as sugar, helps. Finally, the amount of shear one can employ when mixing the lambda into the milk is of paramount importance. Blenders and food processors are excellent, shakers are good, and

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The real problem is that lambda is not available in a pure form or in commercial quantities. As mentioned before, Chondrus contains around 35% lambda. For this reason Chondrus-based products have some cold milk reacting properties, but only about a third of the product is doing all the work. Acicularia/pistillata contains about 70% lambda, but is available only as a byproduct of agar production and not in quantities sufficient to be of much commercial interest. Fractionation of Chondrus is possible but not practical.

Cosmetic Applications. Over the years carrageenan has been used in lotions, creams and even shampoos not so much for its rheological properties which are quite good but rather for its residual properties. By that I mean conditions that cosmetic chemists describe as "finish," "feel" and "rub out." Carrageenan when properly selected and formulated leaves the skin with a soft, smooth finish often described as "velvety" or "quince-like." The problem, in my opinion, has been that carrageenan manufacturers have not understood nor offered cosmetic grade products. On the other hand, cosmetic chemists by and large are suspicious of "natural" products and have not understood carrageenan chemistry sufficiently to achieve the results they are looking for.

A recent development[13] has shown that very low molecular weight (i.e., 10,000-15,000) carrageenan is substantive to hair keratin.

Formulated into a shampoo or conditioner, the carrageenan imparts body to the hair and eliminates the condition called "flyaway," probably by binding moisture and conducting away any static charges which may have been present. Another interesting observation was that the carrageenan appears to be able to bind glycerine to the hair as well. Glycerine and other humectants are not used in shampoos and conditions because they are not substantive and consequently are easily rinsed away. Glycerine has been observed to act synergistically in other ways with carrageenan. That is, in toothpaste formulations, the combination produces significantly higher viscosities than would be otherwise expected. Also, carrageenan will thicken and gel glycerine even when the water content is as low as 10%. The gels, by the way, are very strong and resilient. If low molecular weight carrageenan is substantive to hair keratin, it should also be substantive to less cross-linked skin keratin.

Medical Uses. Graham has reported carrageenan to be effective as a sustained-release agent with certain tranquilizers and hypotensive agents[14]. The aforementioned sols not only suspend but are effective in extending the shelf-life activity of aqueous antibiotic formulas. In the case of a penicillin product, the refrigerated activity level was extended from one to five weeks.

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ulcer therapy. It has been reported that carrageenan taken internally gives very fast and lasting relief to the discomforts of peptic ulcers[16,17]. Three separate mechanisms have been reported. First, a physical protective coating of the hydrated carrageenan is deposited on the ulcerated mucous membrane. Second, the secretion of gastric juices is reduced and, finally, pepsin activity is diminished. A product called Ebimar[24] has been marketed in France for twenty years of more. The reason for its popularity is said to be that the carrageenan treatment does not require the simultaneous use of a bland diet and it is further said that French doctors have given up trying to keep Frenchmen on such a diet. The anti-ulcer properties of carrageenan have also been seriously studied by a number of pharmaceutical companies in this country but the cost and risk in attempting to get an NDA, as well as the fact that it is no longer patentable[17], overshadows any profit potential.

Enzyme Activity. A number of investigators have reported that low molecular weight carrageenan (as well as heparin, chondroitin sulfate, and dextran sulfate) inhibit the activity of the enzyme pepsin[18,19,20]. Gatfield found that lambda carrageenan has an inhibitory effect on trypsin[21] and an enhancement effect on the activity of horseradish peroxidase[22]. Lambda has also been reported by Shipe[23] to inhibit lipase activity in milk. We know that in each case variables such as molecular weight, fraction type, concentration and temperature have a substantial effect on carrageenan-enzyme reactions. Unfortunately, very little is yet known

about this potentially important subject. Researchers for the most part don't sufficiently understand carrageenan chemistry, nor do they have ready access to a range of highly characterized materials. On the other side, carrageenan manufacturers know little about protein chemistry, let alone enzymes. Cooperative efforts will be required to make progress in this important area.

In conclusion, there are many other facets to the carrageenan story which time does not permit us to go into. Some are at this point rather obscure but nonetheless interesting. Others have yet to be made public. In addition, it should be remembered that carrageenan is but one part of the overall red seaweed-hydrocolloid story. Agarose, agar and furcellaran are closely related to carrageenan and have many similar as well as unique properties and applications of their own. For these reasons, and more, carrageenan is surely nature's most versatile hydrocolloid.

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